AMENDMENTS TO THE CLAIMS

1-13. (canceled)

14. (currently amended) A method of producing an amplified DNA fragment comprising

i) amplifying a linear double-stranded or single-stranded DNA by polymerase chain reaction (PCR), using a reaction solution comprising:

a template DNA fragment comprising a double-stranded or single-stranded DNA fragment comprising a sequence encoding a protein or a portion thereof;

a first sense primer that anneals with the 5′ terminal region of the template DNA fragment;

a second sense primer which has a 3′ terminal sequence that is the same as at least a 5′ portion of the first sense primer and a 5′ terminal sequence that is the same as a desired nucleotide sequence; and

an anti-sense primer which anneals with the 3' terminal region of the template DNA fragment;

thereby obtaining ~~an~~ a first amplified DNA fragment;

ii) amplifying the first amplified DNA fragment by polymerase chain reaction (PCR), using a reaction solution comprising:

a) a template mixture comprising

aa) the first amplified DNA fragment,

ab) a second double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 5' terminal region of the first amplified DNA fragment, and

ac) a third double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 3' terminal region of the first amplified DNA fragment;

b) a sense primer which anneals with the 5' terminal region of the second DNA fragment; and

c) an anti-sense primer which anneals with the 3' terminal region of the third DNA fragment;

wherein the second DNA fragment comprises regulatory sequences for transcription and translation of a gene,

and wherein the DNA fragment ac) has a 3'-terminal sequence that is the complement of the 5' terminal sequence of the DNA fragment ab) and the sense primer b) is the same as the anti-sense primer c);

thereby obtaining an amplified DNA molecule comprising the overlapped DNA fragments aa), ab) and ac).

15. (currently amended) A method of producing an amplified DNA fragment comprising amplifying a linear double-stranded or single-stranded DNA by polymerase chain reaction (PCR), using a reaction solution comprising:

a) a template mixture comprising

aa) a first double-stranded or single-stranded DNA fragment comprising a sequence encoding a protein or a portion thereof,

ab) a second double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 5' terminal region of the first DNA fragment, and

ac) a third double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 3' terminal region of the first DNA fragment;

b) a sense primer which anneals with the 5' terminal region of the second DNA fragment; and

c) an anti-sense primer which anneals with the 3' terminal region of the third DNA fragment;

wherein the second DNA fragment comprises regulatory sequences for transcription and translation of a gene, and the concentrations of the second DNA fragment and the third DNA fragment in the reaction solution each range from 5 to 2,500 pmol/L,

and wherein the DNA fragment ac) has a 3'-terminal sequence that is the complement of the 5' terminal sequence of the DNA fragment ab) and the sense primer b) is the same as the anti-sense primer c).

thereby obtaining an amplified DNA molecule comprising the overlapped DNA fragments aa), ab) and ac).

16. (canceled)

17. (previously presented) A method of producing an amplified DNA molecule comprising

i) amplifying a gene segment of interest by polymerase chain reaction, using a reaction solution comprising:

a template comprising a first double-stranded or single-stranded DNA fragment cloned in a vector, said DNA fragment comprising a sequence encoding a protein or a portion thereof;

a first sense primer that anneals with a 5' terminal region of the protein-coding sequence;

a second sense primer which has a 3' terminal sequence that is the same as at least a 5' portion of the first sense primer and a 5' terminal sequence that is the same as a desired nucleotide sequence; and

a first anti-sense primer which anneals with a portion of the vector sequence downstream from the protein-coding region;

thereby obtaining a first amplified DNA fragment; and

ii) amplifying the first amplified DNA fragment by polymerase chain reaction, using a reaction solution comprising:

a) a template mixture comprising

aa) the first amplified DNA fragment,

ab) a second double-stranded or single-stranded DNA fragment comprising a sequence hybridizing with the 5' terminal region of the first amplified DNA fragment, and

ac) a third double-stranded or single-stranded DNA fragment comprising a sequence hybridizing with the 3' terminal region of the first amplified DNA fragment;

b) a third sense primer which anneals with the 5' terminal region of the second DNA fragment; and

c) a second anti-sense primer which anneals with the 3' terminal region of the third DNA fragment;

wherein the DNA fragment ab) comprises regulatory sequences for transcription and translation of a gene, and the concentrations of the DNA fragments ab) and ac) in the reaction solution ii) each range from 5 to 2,500 pmol/L,

thereby obtaining an amplified DNA molecule comprising the overlapped DNA fragments aa), ab) and ac).

18. (previously presented) The method of claim 17, wherein in the amplifying reaction ii) the respective concentrations of the first and second sense primers and of the first antisense primer and the amount of primer dimers produced in the amplifying reaction i) are each less than 20 nmol/L.

19. (previously presented) The method of claim 17, wherein the respective concentrations of primers used for the amplifying reaction i) are from 20 to 500 nmol/L.

20. (previously presented) The method of claim 17, wherein the reaction solution of the amplifying reaction i) comprising the first amplified DNA fragment is diluted 10- to 100-fold before being used in the amplifying reaction ii).

21. (previously presented) The method of claim 17, further comprising a step of removing the first and second sense primers and the first anti-sense primer and primer dimers formed in the amplifying reaction i).

22. (previously presented) The method of claim 17, wherein the amplifying reaction i) is carried out using recombinant microorganisms or a culture broth thereof comprising the template.

23. (previously presented) The method of claim 17, in which the DNA fragment ac) has a 3'-terminal sequence that is the complement of the 5' terminal sequence of the DNA fragment ab) and the sense primer b) is the same as the anti-sense primer c).

24. (currently amended) The method of ~~any one of claims 15 to 17~~claim 15 or 17, wherein the second DNA fragment and/or the third DNA fragment are single-stranded DNA.

25. (currently amended) The method of ~~any one of claims 15 to 17~~claim 15 or 17, wherein the third DNA fragment comprises a transcription termination sequence.

26. (currently amended) The method of ~~any one of claims 15 to 17~~claim 15 or 17, wherein at least one of the second DNA fragment and the third DNA fragment comprises a sequence encoding a tag peptide.

27. (currently amended) The method of any one of claims ~~14 to 17~~14, 15 and 17, in which the desired nucleotide sequence of the second sense primer comprises a sequence encoding a tag peptide.

28. (previously presented) The method of claim 26, wherein the tag peptide is maltose binding protein, cellulose binding domain, glutathione-S-transferase, thioredoxin, streptavidin binding peptide or histidine tag peptide.

29. (previously presented) The method of claim 28, wherein the tag peptide is a histidine tag peptide consisting of the amino acid sequence of SEQ ID No. 1.

30. (previously presented) The method of claim 27, wherein the tag peptide is maltose binding protein, cellulose binding domain, glutathione-S-transferase, thioredoxin, streptavidin binding peptide or histidine tag peptide.

31. (previously presented) The method of claim 30, wherein the tag peptide is a histidine tag peptide consisting of the amino acid sequence of SEQ ID No. 1.

32. (previously presented) A method of producing a protein in a cell-free protein synthesis system comprising:

i) preparing a DNA template for transcription and translation by amplifying a linear double-stranded or single-stranded DNA by polymerase chain reaction (PCR), using a reaction solution comprising:

a) a template mixture comprising

aa) a first double-stranded or single-stranded DNA fragment comprising a sequence encoding a protein or a portion thereof,

ab) a second double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 5' terminal region of the first DNA fragment, and

ac) a third double-stranded or single-stranded DNA fragment comprising a sequence overlapping with the 3' terminal region of the first DNA fragment;

b) a sense primer which anneals with the 5' terminal region of the second DNA fragment; and

c) an anti-sense primer which anneals with the 3' terminal region of the third DNA fragment;

wherein the second DNA fragment comprises regulatory sequences for transcription and translation of a gene, and the concentrations of the second DNA fragment and the third DNA fragment in the reaction solution each range from 5 to 2,500 pmol/L,

thereby obtaining an amplified DNA molecule comprising the overlapped DNA fragments aa), ab) and ac); and

ii) transcribing the amplified DNA molecule *in vitro* to obtain a transcript; and

iii) translating the transcript in a cell-free protein synthesis system to obtain a protein.

33. (previously presented) A method of producing a protein in a cell-free protein synthesis system comprising:

A) producing an amplified DNA fragment comprising

i) amplifying a linear double-stranded or single-stranded template DNA fragment by polymerase chain reaction, using a reaction solution comprising:

the template comprising a first double-stranded or single-stranded DNA fragment comprising a sequence encoding a protein or a portion thereof;

a first sense primer that anneals with a 5' terminal region of the template DNA fragment;

a second sense primer which has a 3' terminal sequence that is the same as at least a 5' portion of the first sense primer and a 5' terminal sequence that is the same as a desired nucleotide sequence; and

a first anti-sense primer which anneals with a 3' terminal region of the template DNA fragment;

thereby obtaining a first amplified DNA fragment; and

ii) amplifying the first amplified DNA fragment by polymerase chain reaction, using a reaction solution comprising:

a) a template mixture comprising

aa) the first amplified DNA fragment,

ab) a second double-stranded or single-stranded DNA fragment comprising a sequence hybridizing with the 5' terminal region of the first amplified DNA fragment, and

ac) a third double-stranded or single-stranded DNA fragment comprising a sequence hybridizing with the 3' terminal region of the first amplified DNA fragment;

b) a third sense primer which anneals with the 5' terminal region of the second DNA fragment; and

c) a second anti-sense primer which anneals with the 3' terminal region of the third DNA fragment;

wherein the DNA fragment ab) comprises regulatory sequences for transcription and translation of a gene, and the concentrations of the DNA fragments ab) and ac) in the reaction solution ii) each range from 5 to 2,500 pmol/L,

thereby obtaining a second amplified DNA molecule comprising the overlapped DNA fragments aa), ab) and ac);

B) transcribing the second amplified DNA fragment *in vitro* to obtain a transcript;

C) translating the transcript in a cell-free protein synthesis system to obtain a protein.